AFDC 3(4792) P3
REV: TZS 269: 1986

DRAFT TANZANIA STANDARD

Ascorbic acid estimation in food and food stuff – Part 1- General method

TANZANIA BUREAU OF STANDARDS

0 Foreword

Ascorbic acid (Vitamin C) is widely distributed in the tissues of all plants and animals, with the exception of the dried seeds of cereals and pulses. It is a vitamin considered vital for the diet required by the body of most organisms at specific requirement level.

The development of this standard was necessitated by the need to establish uniform procedures for quantifying ascorbic acid in foods and food stuffs.

In the preparation of this standard method much considerable assistance was derived from IS: 5838: 1970 (reaffirmed 2015) *Method for estimation of vitamin C in food stuffs,* Published by the Indian Standard institution.

In reporting the results of a test or analysis made in accordance with this standard, if the final value observed or calculated, is to be rounded off it shall be done in accordance with TZS 4:

1.0 Scope

This Tanzania standard specifies method for estimation of Ascorbic acid in food and food stuffs using 2, 6-dichrolophenol indophenols method.

2.0 References

For the purpose of this Tanzania standard the following references shall apply whereby for dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

TZS 59: – *Water for analytical laboratory use – Specification and test method*

TZS 4: -*Rounding off numerical vales.*

3.0 Principle

The hydrogen atoms of the two enolic groups of ascorbic acid can be oxidized readily, as this compound is a strong reducing substance. Measurement of this reducing property of ascorbic acid under appropriate conditions is the basis of several methods for determining the quantity of ascorbic acid.
The compound 2, 6-dichlorophenol indophenol is blue in alkaline conditions and pink in acidic conditions. It is reduced by ascorbic acid to a leukoform. A dilute solution of the dye is added dropwise from a burette to an acid extract of vitamin source. A pink colour that persists for 15 seconds after the addition of one drop of dye is the end point of the titration.

4.0 Apparatus

4.1 100 ml glass stoppered graduated flask

4.2 50 ml Erlenmeyer flasks

4.3 Burette

4.4 Desiccator

4.5 Amber glass stoppered bottle

5.0 Reagents

5 .1Quality of reagents: Unless specified otherwise analytical grade chemicals and distilled water TZS 59(see clause 2) shall be employed.

5.2 Trichloroacetic acid (TCA) - 10% m/m). Dissolve 10 g of TCA in 100ml of water

5.3 Metaphosphoric Acid- 5%

5.4 Standard ascorbic acid solution

Weigh approximately 100 mg of USP (United States Pharmacopoea) ascorbic acid reference standard to ±0.1 mg. Transfer it to a 100 ml glass stoppered graduated flask, dissolve and dilute to the mark with TCA or metaphosphoric acid.

5.5 Standard indophenol solution

Dissolve 50 mg of sodium 2,6 dichlorophenol indophenol, that has been stored in a desiccator over soda lime, in 50 ml of water to which 42 mg of sodium bicarbonate has been added. Shake vigorously and when the indophenol has completely dissolved, dilute to 200 ml of water. Filter the solution through a fluted filter paper into an amber glass stoppered bottle. Keep the bottle stoppered, out of direct sunlight and store in a refrigerator. Decomposition products that make the end point indistinct occur in some batches of dry indophenol and also develop with time in stock solution. Add 5 ml of either TCA or metaphosphoric acid reagent containing excess of ascorbic acid to 15 ml of the indophenol solution. If the reduced solution is not practically colourless discard and prepare a new stock solution. If the dry indophenol dye is proved to be of a bad quality, obtain a new sample.

5.5.1 ***Standardization of indophenol solution***

Standardize the indophenol solution immediately after it has been prepared as follows:

Transfer three 2 ml aliquots of the standard ascorbic acid solution to each of the three 50 ml Erlenmeyer flasks containing 5 ml of either TCA or metaphosphoric acid reagent. Titrate rapidly with the indophenol solution from a 25-ml burette until light but distinct rose pink colour persists for at least five seconds. (Each titration should require approximately 15 ml of indophenol solution and the titration should check within 0.05 ml). In the same way, titrate three blanks composed of 7 ml of either TCA the volume of which is approximately equivalent to the volume of the indophenol solution used in the direct titration.

Calculate and express the concentration of the indophenol solution as milligrams of ascorbic acid equivalent to one millilitre of the indophenol solution. Standardize the indophenol solution daily with freshly prepared standard ascorbic acid solution.

6.0 Procedures

6.1 Preparation of the assay sample

6.1.1 The technique used for preparing the material for analysis is mostly common to every vitamin determination. It should be ensured that the sample taken for the assay is representative of the whole and any deterioration of the vitamin to be examined is prevented.

6.1.2 Powders and liquids should be mixed thoroughly until homogeneity is achieved. Dry materials, such as bread, biscuits and grains, should be ground and mixed thoroughly.

6.1.3 Wet or fresh material may be minced with a knife or scissors or homogenized in a blender, if necessary, in the presence of the extracting solvent.

6.2 Grind an accurately weighed sample (about 5 g) in a mortar with acid washed sand using TCA reagent or metaphosphoric acid and transfer into 100 ml graduated flak. Shake the mixture thoroughly and make up the volume to 100 ml with reagent or metaphosphoric acid. Filter immediately through a fluted paper. The grinding step can be omitted if the material is in powder or liquid form.

NOTE – Interference from phenol and sulphydryl compounds, is diminished by carrying out the reduction below pH value of 4, since most of the phenolic compounds do not reduce indophenol at low pH and reduction by sulphydryl group is so low that a correction can be obtained. Thiosulphates, ferrous and cuprous compounds reduce indophenol and when present in appreciable quantities lead to falsely high levels of ascorbic acid. Another group of inter-fering substance in oxidation reduction methods are the compounds loosely classified together and called reductions. Interference from sulphides, sulphites and thio compounds can be overcome by treatment with formaldehyde. In presence of the substances, the extract is rendered acid to pH 0.6 and formaldehyde is added. This would react with all sulphides, sulphates and thio compounds, leaving the ascorbic acid free. Then the titration in the usual way is carried out (see clause 7). In case of turbid or highly coloured solutions, the estimation can be carried out with the help of a photoelectric colorimeter.

7.0 Determination of Ascorbic Acid

Take 10 ml of the filtrate (see 6.2) and titrate rapidly with indophenol solution. A pink colour that persists for 15 seconds after the addition of one drop of the dye is the end point of the titration. Carry out a blank determination with 11 ml of the reagent along with water sufficient make the volume of the mixture equivalent to 14 ml plus the volume of the indophenol solution required in the direct titration.

8.0 Calculation

Calculate the **Ascorbic Acid** content in the sample as follows:

Ascorbic Acid content, mg per 100 of the sample. = $\frac{A \times B \times 100}{M}$

Where

A = volume in ml of the indophenol solution used for titration
B = mass in mg of the ascorbic acid equivalent to milliliter of the indophenol solution
M = mass in g of the sample taken for the test.

9.0 Repeatability

The difference between the results of a determination in duplicate (obtained simultaneously or in rapid succession but the same analyst) shall not exceed 5%.